Technische Universiteit
Eindhoven
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**iGEM TU/e 2016**
Biomedical Engineering

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# Mutagenesis

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Mutagenesis

# 1 Site-Directed Mutagenesis

**Estimated bench time:** 15 minutes
**Estimated total time:** 2 hours, but much longer if there is a big insert in pET28a
**Purpose:** Introducing a mutation into the plasmid

## 1.1 Materials

- 10X buffer
- Autoclaved $H_2O$
- Bucket with ice
- dNTP mix
- Pair of primers which yield the mutation for the insert
- PCR tubes
- Pipettes and tips
- Plasmid DNA
- QuikChange Lightning Multi enzyme blend
- QuikSolution
- PCR machine

## 1.2 Setup & Protocol

- Keep dNTP mix on ice before addition to reaction mixture.
- Add the components to reaction mixture in the order shown below:

| ***Component*** | **Final Concentration** | **Volume (µL)** |
|---|---|---|
| *$H_2O$* | Add up to 25µL | |
| *10X buffer* | 1X | 2.5 |
| *QuikSolution* | | 0.5 |
| *Plasmid DNA* | 100ng (stock ..ng/µL) | |
| *Primer 1* | 100ng (stock 10 µM) | 1 |
| *dNTP mix* | | 1 |
| *QuikChange Lightening Multi enzyme blend* | | 1 |
| *Total* | | 25 |

- Take the DNA polymerase out of the -20 °C freezer only after all components have been added to the reaction mixture and the PCR machine has been programmed. Do not touch the bottom of the polymerase tube.
- Mix reaction mixture well before start of PCR.
- Run the following PRC program:

| **Step** | **Temperature (°C)** | **Time (sec.)** | **Cycles** |
|---|---|---|---|
| *Initial Denaturation* | 95 | 120 | 1 |
| *Denaturation*<br>*Annealing*<br>*Extension* | 95<br>55<br>65 | 20<br>30<br>30/kb | 30 |
| *Final extension* | 65 | 300 | 1 |
| *Hold* | 4 | | |

# 2 DpnI digestion

**Estimated bench time:** 5 minutes
**Estimated total time:** 65 minutes
**Purpose:** Digestion of the template plasmid from the PCR product mixture.

## 2.1 Materials

- DpnI restriction enzyme
- Bucket with ice
- PCR product
- PCR machine

## 2.2 Setup & Protocol

- Thaw the DpnI restriction enzyme on ice.
- Add 1µL of DpnI restriction enzyme directly to each amplification reaction.
- Incubate for 1 hour at 37 °C, in PCR machine.

# 3 Transformation into E. Coli XL10-GOLD

**Estimated bench time:** 40 minutes
**Estimated total time:** 2.5 hours
**Purpose:** Placing the plasmid into bacteria for amplification.

For more information, see our general Transformation into E. Coli XL10-GOLD protocol.

# 4 Plating cells on agar

**Estimated bench time:** 15 minutes
**Estimated total time:** 18 hours
**Purpose:** Amplification of the ligation product.

For more information, see our general Plating protocol.

- Be sure to dilute the bacteria 10x with SOC!
- SOC : bacteria = 10:1 - you have 50 µL bacteria, so add 500 µL SOC
- Plate 100 µL